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Dichotomous Effects of Cadmium and Selenium on Erythropoiesis in Mice

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A number of complex interrelationships exist among trace metals that suggest synergistic, additive, or inhibitory effects of the test metals (Fassett 1975, Levander 1977). Cadmium is one such material that has received considerable attention because of its link to a wide spectrum of pathophysiological conditions including those that are directly or indirectly associated with the production and release of erythrocytes, i.e., erythropoiesis. It has been reported that cadmium induces pulmonary surface degeneration (Smith et al. 1976), decreases the half time of erythrocytes (Berlin and Friberg 1960), increases total blood volume (Berlin and Piscator 1961), promotes hypertension (Perry 1976), and causes a severe anemia (Fox et al. 1971). The toxicity of selenium is highly diverse (Ganther 1965). Similarily in relation to erythropoiesis, selenium adversely affects the liver (Diplock et al. 1971), depresses hemoglobin content (Jacobs and Frost 1981), and effects the development of not only a hypochromic anemia (Nagai 1959) but also a hemolytic one (Halverson et al. 1970). For the reported anemias that were induced by cadmium and selenium, exposure time was chronically protracted. The investigations reported here were designed to test the effects of a single acute non-lethal treatment of salts of cadmium, selenium and a combination of the two, cadmium selenite, on the time course and extent of erythropoiesis. Peripheral erythrocyte production and splenic erythropoietic activity were used as indices of erythropoiesis. Concerning the latter parameter, the spleen has been shown (Gresham et al. 1971) to be a key organ for erythropoiesis in mice.

MATERIALS AND METHODS

Female mice of the ICR strain were isolated, quarantined for two weeks, and randomized into experimental and control groups. Unless otherwise designated five animals comprised each treatment and control group; standard rodent chow and water were freely available at all times. At time 0 the following solutions were injected (ip): aqueous sodium selenite, cadmium chloride, and cadmium selenite (Pfaltz and Bauer, Stanford, CN 06902). All dosages were 2.5 mg/kg in a 0.2 ml volume.

Control mice were injected with 0.2 ml isotonic saline. A standard volume was used for all treatments since weight variations among animals were relatively small. Weight ranged between 22 and 28 g with a mean weight of 26.4 g. At designated intervals following injection, mice were sacrificed by cervical dislocation with cardiac blood withdrawn using sodium heparin as the anticoagulant. Prior to this animals were injected with 0.5 μCi radioiron (^{59}Fe) citrate (aqueous, specific activity = 9.45 mCi/mg, NEN Products, Boston MA 92118). Radioiron was given 24 hr before sacrifice for those groups for which the collection times were 1,2,3, or 4 days after the initial treatment. For the 3,6, and 12 hr collection groups ^{59}Fe was administered immediately after the test material. Thus, for all groups except the 3,6, and 12 hr ones, 24 hr ^{59}Fe incorporation percentages into peripheral erythrocytes were determined. Three, 6, and 12 hr incorporation percentages were calculated for the earlier times. Such utilization of radioiron, relative to the total ^{59}Fe activity administered, is known to be a specific index of the rate and extent of erythropoiesis (Lajtha 1961). Comparisons of percentages incorporation were made among the test groups and controls using analysis of variance. A deep well scintillation counter was employed to detect radioactivity with appropriate corrections made for critical counting parameters using the method described by Hodgson (1962). Before a blood sample was counted, the cells were rinsed in 4 ml chilled isotonic saline and centrifuged to obtain an erythrocyte pellet. The supernatant containing the plasma was discarded. For each blood sample, the packed red blood cell volume, i.e., hematocrit (Hct), was measured by a micromethod. Brilliant cresyl blue (3%) was used to stain peripheral blood smears that were subsequently counterstained with Wright's stain in order to determine the percentage of reticulocytes present in peripheral blood. The criteria for differentiating reticulocytes from other cells conform to those given in Bessis (1977). Immediately after sacrifice, spleens were dissected, rinsed three times with chilled saline, blotted dry, weighed to the nearest mg, and counted for their radioactivity. The resultant counts were then used for the expression of percentage ^{59}Fe incorporation into 100 mg spleen. Others (Morse et al. 1972) have found that certain trace metal precipitate at the site of injection. Thus, mice were examined at the time of sacrifice for abdominal lesions associated with injections.

RESULTS AND DISCUSSION

Within minutes following injections of cadmium chloride or cadmium selenite, mice developed muscular spasms of the lower limbs and entered a semi-comatosed state that lasted for approximately half an hour. These effects appeared to be only temporary and did not influence later food intake or water consumption. Dosages of the cadmium and selenium compounds greater than those employed for these studies were lethal to test animals. It is assumed, therefore, that the 2.5 mg/kg level of treatment represented the maximum tolerable dose. No necrotic tissues or lesions were observed.

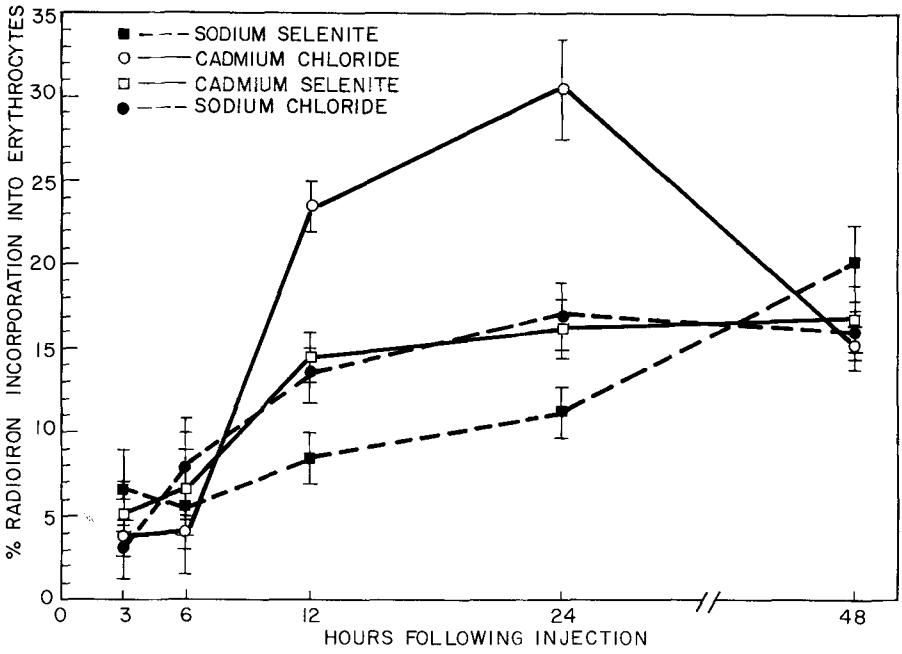

Figure 1. Radioiron incorporation into erythrocytes from mice injected with sodium selenite, cadmium chloride, cadmium selenite, and sodium chloride (time 0). Vertical lines represent standard errors of the means (SEM).

Figure 1 illustrates the results of the erythrocyte radioiron incorporation studies. It is noted there are no statistically significant differences (analysis of variance) among groups at 3 or 6 hr post-injection. For the 12 hr cadmium chloride- and sodium selenite-treated groups, the mean percentages, however, did deviate significantly (analysis of variance, $p < 0.05$ and 0.10, respectively) from both the cadmium selenite and sodium chloride groups' mean percentages. The elevated cadmium chloride and depressed sodium selenite percentages were also apparent at the 24 hr collection time. Two days after treatment, all test groups showed percentages that clustered around the mean percentage of the control, i.e. about 16%, with no significant differences between any two groups. This was the case for the 3 and 4 day collection intervals with mean percentages for all four groups ranging from a mean high of 18.3 ± 1.4 (SEM) for the cadmium selenite-treated mice to a mean low of 14.9 ± 2.0 (SEM) for the sodium selenite-injected females.

The splenic radioiron incorporation percentages for the three experimental groups did not differ significantly from those the control group at any of the test periods. Mean control spleen percentages ranged between 2.2 ± 0.19 (SEM) and 3.3 ± 0.37 (SEM) for days 1 and 4, respectively, which markedly overlapped those

mean percentages of the three test groups. In addition, there were no statistically significant differences in percentage hematocrits observed at 3,6,12, or 24 hr and 2,3, or 4 days after injection of the test salts compared to hematocrit values of the controls at comparable times. Hematocrit percentages for all groups ranged between 36.3 ± 2.1 (SEM) and 40.7 ± 3.1 (SEM). Similarily, reticulocyte counts did not vary significantly among the three experimental groups or between the experimental and control groups. The mean reticulocyte percentages for the 3,6, 12, and 24 hr collection times for sodium selenite-, cadmium chloride-, cadmium selenite-, and sodium chloride-treated mice were, respectively, 1.2, 1.4, 1.0, and 1.9%; 1.1, 1.3, 1.4, and 2.0%; 1.6, 0.9, 0.9, and 1.3%; and 1.2, 1.0, 2.2, and 1.8%. The slides used to score for the reticulocyte percentages revealed no apparent deviation from normal erythrocyte or reticulocyte cytology for the cadium chloride, sodium selenite, or cadmium selenite groups.

Results indicate that the effects of cadmium chloride and sodium selenite on erythropoiesis in female mice occur very late in the total process. It has been estimated that it takes about four days for erythropoiesis to be completed from the earliest erythroid stem cell to the final cellular product, the erythrocyte (Byron, 1972). Thus, the stimulatory effect of cadmium and the inhibitory effect of selenium or radioiron incorporation are manifest during the last 12 to 24 hours of the cellular progression. Because of the time course of the effects it is tempting to postulate that both salts have a common target, one that selenium renders less effective in its contribution(s) to erythropoiesis and that cadmium exerts a reciprocal effect. This postulate is supported by the fact that when both cadmium and selenium are present together as cadmium selenite, no effect is detected, i.e., radioiron incorporation percentages parallel those of the control percentages at 12 and 24 hr following injection.

Prolonged treatment of selenium has been shown to induce a hemolytic anemia (Halverston et al. 1970). The transient erythropoietic depression as reported here was not due to erythrocyte hemolysis, since hematocrit values of sodium selenite-treated mice were essentially the same as the sodium chloride-injected controls. Chronic exposure to selenium has been reported to create a hypochromic anemia in female humans (Nagai 1959). The single treatment of selenite administered in there studies did not have such an effect, although it promoted a reduced radioiron incorporation into hemoglobin. It is possible, that with repeated selenium treatment, hemoglobin synthesis could be continually dampened to induce a condition of hypochromic anemia. Although not specified, the anemia induced by cadmium as reported by Fox et al. (1971), appeared to be one of a microcytic hypochromic nature. Data reported here show an acceleratory action of cadmium on erythropoiesis rather than on deceleratory one. This

stimulatory effect is not due to compensation for a reduced oxygen carrying capacity of peripheral erythrocytes because of a reduced erythrocytic size and hemogloblin content, because the packed red blood cell volumes of cadmium chloride-injected mice and those of control mice did not significantly differ during any experimental period. Perhaps, with a chronic administration of cadmium, erythroid tissue may undergo hypoplasia due to prolonged and continued stimulation, thus, resulting in a reduction of erythropoiesis and subsequently creating an anemic state. Percentages of reticulocytes for all treatments and controls were in the normal range and did not vary significantly even though the radioiron incorporation values did. It would appear then that both cadmium and selenium exert their reciprocal effects on erythropoiesis at the latter hemoglobin synthesizing stages and not by affecting the rate of cellular progression through the latter stages, i.e., the last 12 to 24 hr. Such differences in radioiron utilization and reticulocyte responses have been noted elsewhere (Hogan 1977).

It is of interest that the dichotomous effects of cadmium and selenium on radioiron incorporation into peripheral erythrocytes are lacking in splenic tissue. This may be associated with a larger number of vulnerable sites to cadmium and selenium in bone marrow as opposed to spleen. In addition, splenic erythropoiesis is thought to function as a "back-up" system for the primary erythrocyte production site, bone marrow (Gresham et al. 1971). Thus, the inhibitory influence of selenium would be more likely to be detected as opposed to cadmium's stimulatory effect. Since the suppressor action of selenium is relatively short-lived, splenic tissue may not be affected beyond its normal, control level of activity.

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